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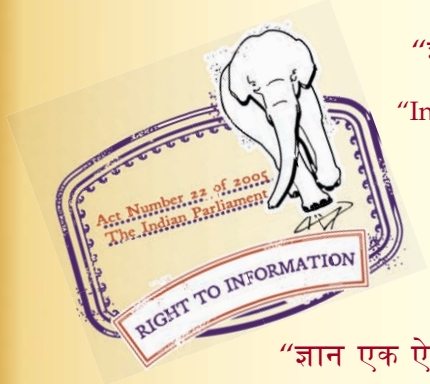


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IS : 7203 - 1973
(Reaffirmed 2005)

*Indian Standard*

# SPECIFICATION FOR CASEIN HYDROLYSATE ( ACID DIGESTED ), MICROBIOLOGICAL GRADE

UDC 577·152:663·1



INDIAN STANDARDS INSTITUTION
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110001

*September* 1974

# *Indian Standard*

# SPECIFICATION FOR CASEIN HYDROLYSATE ( ACID DIGESTED ), MICROBIOLOGICAL GRADE

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# *Indian Standard*

# SPECIFICATION FOR CASEIN HYDROLYSATE ( ACID DIGESTED ), MICROBIOLOGICAL GRADE

## 0. FOREWORD

**0.1** This Indian Standard was adopted by the Indian Standards Institution on 26 November 1973, after the draft finalized by the Food Hygiene, Sampling and Analysis Sectional Committee had been approved by the Agricultural and Food Products Division Council.

**0.2** Unless the ingredients used in media for microbiological analysis are of uniform quality, the results obtained might be erroneous and might be unreliable. Since the media used in different laboratories often differ greatly in their quality, the results of microbiological analysis at different laboratories cannot be compared. Therefore, with a view to unifying the practices of different laboratories dealing with microbiological work and providing guidance to the indigenous manufacturers regarding the quality, it was decided to bring out a series of Indian Standard specifications for ingredients commonly used in media for microbiological analysis.

**0.3** Casein hydrolysate ( acid digested ) microbiological grade, is used for microbiological work especially for growing whooping-cough organism in large quantities for production of vaccine. It is a yellowish white fine powder, highly soluble in water but insoluble in alcohol, ether and chloroform.

**0.4** For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test, shall be rounded off in accordance with IS: 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

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## 1. SCOPE

**1.1** This standard prescribes the requirements and the methods of tests for casein hydrolysate ( acid digested ) microbiological grade.

*Rules for rounding off numerical values ( *revised* ).

## 2. REQUIREMENTS

**2.1** A one percent solution in water shall show colourless to light straw yellow liquid having a *p*H between 6·0 and 7·0.

**2.2** It shall be able to support growth of *Bordetella pertussis* when tested according to method shown in Appendix A.

**2.3 Iron** — It shall pass the test prescribed in Appendix B.

**2.4** The material shall also conform to the requirements given in Table 1.

TABLE 1 REQUIREMENTS FOR CASEIN HYDROLYSATE

| Sl No. | Characteristic | Requirement | Method of Test | | |
|---|---|---|---|---|---|
| | | | Ref to Appendix of this Standard | Ref to Clauses of IS: 6854-1972* | Ref to Appendix of IS: 7128-1973† |
| (1) | (2) | (3) | (4) | (5) | (6) |
| i) | Moisture content, percent by mass, *Max* | 7 | — | 4 | — |
| ii) | Total nitrogen, percent by mass, *Min* | 8 | — | 9 | — |
| iii) | Amino nitrogen, percent by mass, *Min* | 6 | — | 8 | — |
| iv) | Sodium chloride, percent by mass, *Min* | 30 | — | 11 | — |
| v) | Ash, percent by mass, *Min* | 35 | — | 6 | — |
| vi) | Ether soluble extract, percent by mass, *Max* | 0·1 | C | — | — |
| vii) | Phosphorus (as P), percent by mass, *Max* | 0·9 | — | — | E |

*Method of sampling and test for ingredients used in media for microbiological work.

†Specification for proteose peptone, microbiological grade.

## 3. PACKING, STORAGE AND MARKING

**3.1 Packing** — The material shall be securely packed in well-filled amber coloured moisture proof wide mouth containers with tight fitting lids.

**3.2 Storage** — The material shall be stored in a cool and dry place.

**3.3 Marking** — Each container shall be marked legibly to give the following information:

a) Name of the material including the words 'Microbiological grade',

b) Name and address of the manufacturer,

c) Minimum net content,

d) Batch or code number, and

e) The label should indicate quantity of sodium chloride, total nitrogen and phosphate. It shall also indicate that the material passes the prescribed test for iron.

**3.3.1** The container may also be marked with the ISI Certification Mark.

NOTE — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution (Certification Marks) Act and the Rules and Regulations made thereunder. The ISI Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard under a well-defined system of inspection, testing and quality control which is devised and supervised by ISI and operated by the producer. ISI marked products are also continuously checked by ISI for conformity to that standard as a further safeguard. Details of conditions under which a licence for the use of the ISI Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

## 4. SAMPLING

**4.1** The representative samples of the material shall be drawn according to the method prescribed in 3 of IS:6854-1972*.

## 5. TESTS

**5.1** Tests shall be carried out by the methods prescribed in **2.2** and in col 4, 5 and 6 of the Table 1.

**5.2 Quality of Reagents** — Unless specified otherwise, pure chemicals and distilled water ( *see* IS:1070-1960† ) shall be employed in the tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

*Methods of sampling and test for ingredients used in media for microbiological work.

†Specification for water, distilled quality ( *revised* ).

# APPENDIX A

( *Clause* 2.2 )

## TEST FOR ABILITY TO SUPPORT GROWTH

### A-1. GROWTH SUPPORTING PROPERTIES

**A-1.1 Strain** — *Bordetella pertussis* strain No. 134 or 509 ( Dutch strain ) or any other suitable strain in phase I approved by an International authority. Maintain the strain in such a way that the organism is retained in phase I.

### A-2. BORDET-GENGOU MEDIUM

**A-2.1 Base Medium** — Prepare the medium with 12·5 percent peeled and cleaned potato slices, 0·45 percent sodium chloride, 1·0 percent glycerol, 1·0 percent proteose-peptone ( *see* IS : 7128-1973* ) and 2·25 percent agar ( *see* IS : 6850-1973† ) in distilled water. Cut into thin slices the peeled and cleaned potatoes and boil with sodium chloride in one-fourth the amount of distilled water until the potato slices fall to pieces. Make up the water lost during boiling and filter through linen and adjust the reaction to *p*H 7·0. Dissolve the agar separately by boiling in the remaining amount of distilled water and to this add the boiled and filtered potato extract, glycerol and proteose-peptone and mix well. Distribute into bottles and sterilize by steaming at 100°C for one hour followed by autoclaving at 115°C for 10 minutes.

**A-2.2 Complete Medium** — Prepare the complete medium with the 2 volumes of base medium ( **A-2.1** ) and 1 volume of sterile defibrinated horse blood. Melt the base medium ( **A-2.1** ) by steaming for one hour at 100°C mixing well during heating. Place in a water bath at 55°C until the temperature of the liquid base medium drops to about 65°C. Warm sterile defibrinated horse blood in the water bath at 55°C for 2 to 3 minutes and add to the liquid base medium. Mix well and pour at least 30 ml into sterile petri dishes of 10 cm diameter.

### A-3. TEST PROCEDURE

**A-3.1** Prepare medium with the following composition:

| | |
|---|---|
| Casein hydrolysate ( Acid digested ), percent | 1·4 |
| Potassium chloride ( KCl ), percent | 0·02 |

*Specification for proteose-peptone, microbiological grade.

†Specification for agar, microbiological grade.

| | |
|---|---|
| Monobasic anhydrous potassium phosphate ( $KH_2PO_4$ ), percent | 0·05 |
| Magnesium chloride ( $MgCl_2$, $6H_2O$ ), percent | 0·01 |
| Nicotinic acid, percent | 0·002 |
| Glutathione, percent | 0·001 |
| Starch, percent | 0·1 |

Distilled water

Distribute in 100 ml amounts into Roux bottles and sterilize by autoclaving at 115°C for 20 minutes.

**A-3.2** Grow the strain of *B. pertussis* on a Bordet-Gengou plate ( **A-2.2** ) at 35°C for 72 hours and subculture on to another Bordet-Gangou plate. Incubate at 35°C for 48 hours and emulsify the growth in 5 ml of sterilized solution of 1 percent casein hydrolysate ( acid digested ). Inoculate 0·1 ml of the suspension into 100 ml of the medium as prepared in **A-3.1.** Incubate at 35°C for 40 to 48 hours taking care to lay the Roux bottle on its side so as to expose a large surface. Make two further serial subcultures into the medium as prepared in **A-3.1** using 1 ml as the inoculum for these serial subcultures. Each time incubate at 35°C for 40 to 48 hours exposing a large surface of the medium. Transfer the entire contents of the last subcultured and 40 to 48 hours incubated Roux bottle of medium ( **A-3.1** ) to suitable containers. Centrifuge at 2 500 rpm for 40 minutes. Wash the deposits with saline and pool into one container and centrifuge again. Suspend the deposit in 15 ml of 0·85 percent saline.

## A-4. REQUIREMENTS

**A-4.1** The saline suspended material as obtained from **A-3.2** shall meet the following requirements:

a) *Bacterial Density* — It shall have a bacterial density of more than $2 \times 10^{10}$ organisms per ml when determined by the following procedure:

'Not less than $\frac{1}{2}$ dilution of the suspended material diluted with physiological saline should match, when inspected visually, with the WHO International Opacity Reference Preparation containing 10 units of opacity which is equivalent to $1 \times 10^{10}$ organisms per ml.

b) *Phase Variations* — It shall show profuse growth of *B. pertussis* when inoculated on Bordet-Gengou plate and no growth on ordinary nutrient agar medium.

# APPENDIX B

( *Clause* 2.3 )

## LIMIT TEST FOR IRON

### B-1. REAGENTS

**B-1.1 Casein Hydrolysate Solution** — Dissolve 2·3 g casein hydrolysate in 50 ml of distilled water. Make up the volume to 100 ml in a volumetric flask.

**B-1.2 Hydrochloric Acid, Dilute** — Dilute 250 ml concentrated hydrochloric acid ( sp gr 1·18 ), reagent grade, to 1000 ml with distilled water.

**B-1.3 Standard Iron Solution** — Dissolve 0·173 g ferric ammonium sulphate, reagent grade, in 100 ml distilled water. Add 5 ml dilute hydrochloric acid and make up the volume to 1 000 ml in a volumetric flask. This solution contains 0·02 mg iron in 1 ml.

**B-1.4 Ammonia Solution, Dilute** — Dilute 375 ml of ammonia solution ( sp gr 0·88 ), reagent grade to 100 ml with distilled water.

**B-1.5 Citric Acid** — 20 percent solution.

**B-1.6 Thioglycollic Acid**

### B-2. PROCEDURE

**B-2.1** To three, 50 ml capacity Nessler cylinders, labelled Blank, Standard and Casein hydrolysate respectively, add 40 ml of distilled water to the blank, 2 ml of standard iron solution diluted to 40 ml with distilled water to the standard and 35 ml of casein hydrolysate solution to the casein hydrolysate cylinders.

**B-2.2** Add in succession to each cylinder, 2 ml of citric acid solution and 2 drops of thioglycollic acid, and mix well.

**B-2.3** Make the solutions alkaline to about *p*H 8 using *p*H test paper with dilute ammonia solution, make up the volumes to 50 ml with distilled water and mix well.

**B-2.4** Allow the solutions to stand for 5 minutes. The colour produced by casein hydrolysate should not be more intense than the colour produced by the standard iron solution. The blank should be free of any tinge of pink colour.

# APPENDIX C

[*Table* 1, *Item* (vi)]

## DETERMINATION OF ETHER SOLUBLE EXTRACT

### C-1. REAGENT

**C-1.1 Diethyl Ether** — anhydrous.

### C-2. PROCEDURE

**C-2.1** Extract 2 g of the ground material in a continuous extraction apparatus with diethyl ether for 18 hours. Remove the ether by distillation, followed by heating the flask on a boiling water-bath. Dry the residue in an oven at 110 C $\pm$1°C till the loss in mass between two successive weighings is less than 2 mg. Shake the residue with 2 to 3 ml of diethyl ether at room temperature, allow the residue to settle and decant the ether. Repeat the extraction until no more of the residue dissolves. Dry the flask again until the loss in mass between two successive weighings is less than 2 mg. Note the lowest mass.

### C-3. CALCULATION

$$\text{Non-volatile ether extract, percent by mass} = \frac{100\,(W_1 - W_2)}{W}$$

where

$W_1$ = mass in g of the flask with the non-volatile ether extract,

$W_2$ = mass in g of the flask with the ether insoluble residue after decantation, and

$W$ = mass in g of the material taken for the test.

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5404-1969 Code of practice for handling of food samples for microbiological analysis

5835-1970 Method for estimation of vitamin D in foodstuffs

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5838-1970 Method for estimation of vitamin C in foodstuffs

5839-1970 Code for hygienic conditions for manufacture, storage and sale of ice-creams

5886-1970 Method for estimation of carotenes and vitamin A ( retinol ) in foodstuffs

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6542-1972 Code for hygienic conditions for fruit and vegetable canning units

6850-1973 Agar, microbiological grade

6851-1973 Meat extract, microbiological grade

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Printed at Britannia Calendar Mfg Co, Calcutta, India